Technology evaluation: Sch-58500, Canji

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Sch-58500 is a gene therapy utilizing the p53 gene and is under development by Canji and Schering-Plough for the potential treatment of various types of cancer. It is in phase II/III clinical trials in the US for stage III ovarian cancer [328228,328893], phase II clinical trials for hepatocellular and colorectal cancer metastatic to the liver [273331,324279], and phase I clinical trials for several other types of cancer [282801,284932,328228].

Originator Canji Inc

Licensees Genzyme Molecular Onclology, Schering-Plough Corp

Status Phase III Clinical

Indications Carcinoma, liver tumor, breast tumor, colorectal tumor, lung tumor, leukemia, head and neck tumor, melanoma, ovary tumor, neoplasm

Action Anticancer

Technology Gene therapy

Synonyms ACN-53; ACN-p53 TSG; Ad-p53; rAd/p53, Canji/Schering-Plough; gene therapy (p53), Canji/Schering-Plough

Introduction

Canji/Schering-Plough are currently evaluating a new antitumor gene therapy agent, Sch-58500. It is a recombinant adenovirus (Ad) expressing a human p53 apoptosis-inducing gene that triggers targeted tumor cells to undergo programmed cell death (apoptosis). Tumors that are especially sensitive to this type of therapy are those in which the normal cellular *p53* gene is not functional. For example, non-functional (mutant) p53 is expressed in 50% of patients with primary breast tumors and their prognoses are significantly more grim than patients with breast tumors with functional *p53* genes [255115]. Approximately 50% or more of other types of tumors have a non-functional *p53* gene, which suggests that gene therapies such as Sch-58500 that could target p53 mutant cancer cells might have wide utility in the treatment of cancers. The virus has been designed to infect susceptible cells, which include many types of tumor cells [246633,359068]. By doing so, the virus causes the infected cell to transcribe the *p53* gene and translate the message to a functional protein product that will cause the tumor cell to undergo apoptosis [246632,284805]. Because many cancer cell lines were inhibited in cell culture, Sch-58500 is now being tested in clinical trials in patients with a variety of tumors.

Vector

The vector of the *p53* gene, Ad type 5 (Ad5) is a replication-deficient virus, which lacks the genes needed for infectious virus replication, ie, *p9*, *E1*, *E1A* and *E3* [168287]. As a result, the virus can infect a cell but cannot direct that cell to replicate infectious virus progeny. However, the virus can deliver a functional *p53* gene to any cell to which it can attach and penetrate. Once uncoated, the *p53* gene can be transcribed and translated into a functional gene product that will trigger an apoptotic pathway. The *p53* gene was inserted into the Ad genome, along with a cytomegalovirus promoter that facilitates efficient transcription of the *p53* gene [284805].

Pharmac logy

The antitumor activity of Sch-58500 probably results from several modes of inhibition. Firstly, the vector delivers the *p53* gene to any cell that it can infect. The subsequent transcription and translation of the *p53* gene results in an apoptosis-inducing protein product leading to programmed cell death [246630,284932]. Secondly, specialized immune cells, called natural killer cells, are activated at the site of the tumor, which destroy the tumor cells infected with virus vector and some 'bystander cells' at the site of virus infection [380033]. Thirdly, the vector itself mediates tumor growth suppression [168287]. In addition, animal studies have demonstrated that vector also reduces the spread or metastatic potential of the tumor cells that become infected with the vector [255115,284805]. In addition, Sch-58500 increases the sensitivity of tumor cells to inhibition by chemotherapeutic agents such as 5-fluourouracil, cisplatin, etoposide, doxorubicin and paclitaxel (National Institutes of Health) [380035]. In tumor-bearing animals, paclitaxel acted synergistically with Sch-58500 to kill tumors. The mode of action of this synergism appeared to be an enhancement of the uptake of Sch-58500 into the tumor cells, thereby increasing the effective p53 concentration [284304].

The vector is usually administered in the dose range of 1×10^{9} to 7.5×10^{12} total virus particles, depending on the type of tumor [308167]. The mode of delivery is also tumor-dependent, eg, it can be directly injected into brain, head or neck tumors, or intraperitoneally for ovarian cancers, or via the hepatic artery for liver cancers [339443,380035,380041]. The vehicle for delivery is often normal saline. When injected, the vector can penetrate one to ten cell layers deep, depending on the nature of the tissue [380042].

Sch-58500 reduced the spread of certain tumors in immunocompromised mice by 60 to 80% [255115]. The importance of tumor growth suppression of the Ad vector itself was demonstrated in mice that were treated with the potent immunosuppressant, dexamethasone. Tumor growth was still suppressed by the p53-mediated mechanism, but not by the virus-mediated suppression of growth [380033]. Suppression of NK cell response with antibody that neutralized NK cells gave the same result, ie, inhibition of

NK-mediated cell destruction but no effect on p53-induced programmed tumor cell [380033].

Combination therapy with other antitumor drugs has also been evaluated in cell culture. Sch-58500 was used in combination with Sch-66336 (Schering-Plough Research Institute). The latter compound is a farnesyl transferase inhibitor that inhibits the addition of a farnesyl group on RAS proteins, which is an intermediate in an apoptosis pathway [325867,359068]. This combination was a more potent antitumor therapy than either drug alone. Greater efficacy of inhibition was achieved against DU-145 human prostate and *wap-ras/F* transgenic mouse cancer models for Sch-58550 in combination with other agents [380033]. The combination of Sch-58500, cisplatin and paclitaxel was particularly potent in an ovarian cancer animal model. Additionally, Sch-58500 in combination with FDA approved drugs such as cisplatin, doxorubicin, 5-fluorouracil, methotrexate and etoposide resulted in a more potent suppression of tumor cell proliferation in SSC-9, SSC-15 and SSC-25 head and neck tumor cells, SK-OV-3 ovarian tumor cells, DU-145 prostate tumor cells, MDA-MB-468 and MDA-MB-231 breast cancer cells [168287]. Greater anticancer activity was also observed when four human tumor xenografts growing in mice were treated with these combinations [380035]. Sch-58500 was also shown to be safe and efficacious in preventing tumor growth of a human colorectal adenocarcinoma in *nu/nu* immunodeficient mice without thymuses (reduced cellular immunity) [246635]. The mechanisms whereby a synergistic or additive effect was achieved may be due to one drug enhancing the uptake of another, or one drug enhancing the sensitivity of the tumor cells to inhibition by another drug. For instance, it was found that the paclitaxel enhanced the uptake of Sch-58500 [284304], and that Sch-58500 rendered certain tumor cells susceptible to the other drugs used in the combination therapy [284304].

Toxicity

Sch-58500 was generally well tolerated. However, there are concerns about the adverse effect of similar Ad vectors, arising from the death of a patient receiving gene therapy for ornithine transcarbamylase deficiency (OTCD). This has led the FDA to request discontinuation of patient enrollment in several clinical studies of Sch-58500 as a precautionary measure until the reason for patient death is determined [343174,343175].

Clinical Development

Phase I

A phase I/II clinical trial was carried out to evaluate the efficacy of Sch-58500 in recurrent ovarian cancer [380044], but this has since been discontinued. Another phase I study was used to determine the safety and efficacy of Sch-58500 in patients with advanced head and neck cancer [339443]. In this study, Sch-58500 was administered by single or multiple injection to patients with tumors amenable to injection therapy. They received four dose levels of virus based on total virus particles injected. In preliminary results, functional p53 DNA was detected in tumors by reverse transcriptase-polymerase chain reaction (RT-PCR) in four of ten patients whose results were available [339443].

A phase I trial was carried out in 64 patients with hepatic metastases of colon cancer, head and neck cancer, ovarian canc r and melanoma [284304]. Transgenic expression of p53 was detected by RT-PCR and was dose-dependent. Antibody against the Ad vector was also detected, although it did not influence the expression of p53 within the tumor. In another phase I trial involving 62 patients (including those with the tumors described above, as well as patients with non-small-cell lung carcinoma), 30 of 57 patients expressed normal p53 at the tumor site [282801].

70 Patients with brain cancer, head and neck cancer and ovarian cancer were enrolled in an efficacy trial [284932]. Those with the head/neck and brain cancers received 10^9 to 10^{12} virus particles in one administration. Biopsies were taken from 69 of the patients, and in 33 the p53 transcript was detected in a dose-responsive manner. The more particles that were given, the larger amounts of p53 were detected.

In a trial to determine toxicity, gene expression and immune response in patients with primary metastatic liver cancer or ovarian cancer, Sch-58500 was administered by hepatic artery injection or intraperitoneally, respectively [306641]. This was a single/multidose trial either with Sch-58500 alone or in combination with traditional anticancer agents. Antibody to the virus vector was detected but did not affect the dose-dependent expression of p53 at the tumor site. Some mild to moderate side effects were observed, which included fever, malaise and anemia. In another study, 30 patients with hepatocellular carcinoma received a single dose of 7.5 x 10^9 to 7.5 x 10^{13} virus particles [312158]. Here, moderate toxicity was noted, which included the standard symptoms described previously, along with tachycardia and hypertension. Greater expression of p53 was detected at the tumor site than in the normal cells, although the apoptotic index was the same on both the treated side of the liver containing the tumor cells and the non-treated side of the liver. This indicated that normal cells were not adversely affected by the treatment and that tumor cells not previously expressing p53 did express p53.

A combination, single/multiple dose study was undertaken in 41 ovarian cancer patients [312158]. Patients receiving one dose were injected with 10^{10} or 10^{12} particles, and those receiving multiple doses received 10^{12} to 10^{13} virus particles. The recipients of the combination therapy were injected with platinum ip, or with taxol/carboplatin iv. There were moderate side effects, including nausea and vomiting, and p53 was detected at the tumor site. 80 Patients with head and neck tumors received single and multiple doses of Sch-58500 (10^9 to 10^{13} virus particles) and combination therapy via several routes of inoculation [328228]. The maximum tolerated dose in these patients was dependent on the route of administration, with ip administration giving best results. When administered iv, some lymphopenia and thrombocytopenia were detected. Vector shedding also occurred.

Another phase I ovarian cancer study using single and multiple dosing regimens and combination therapy was initiated in 36 patients [347524]. For this study, drugs were administered ip; although moderate toxicity was noted

(nausea, fatigue, hypotension, fever and anemia), there was a reduction in CA-125 tumor marker in 54% of the patients.

Phase II/III

A number of phase II and phase II/III clinical trials have been planned or have commenced. A phase II study in patients with primary or metastatic liver tumors begun in 1998 [328228]. These patients received multiple high doses of Sch-58500 alone or in combination with traditional antitumor agents, either via the hepatic artery or ip; this study has now been discontinued

A phase II study was carried out to determine the efficacy of Sch-58500 in combination with traditional chemotherapeutic drugs in patients with liver and colorectal cancers. The patients were implanted with a pump to deliver the standard drug therapies, such as FLDTR, dexamethasone and Leucovir. In 1999, the FDA asked Canji and Schering-Plough to cease this trial temporarily because of safety concerns that arose from an unrelated trial in which another company was testing an Ad vector and death of a patient had occurred [343174,343175].

A multicenter phase II trial of Sch-58500 in patients with colorectal cancer metastatic to liver commenced in May 1999, along with a trial in patients with hepatocellular cancer metastatic to liver [324279]. Several phase II/III studies were started in 1999 and 2000, including study of the ffect of common chemotherapeutic agents used alone or in combination with Sch-58500 ip in newly diagnosed ovarian cancers with the *p53* mutation [328228,328893].

Side Effects and Contraindications

Most trials report few side effects with Ad-p53 when used alone, relative to the adverse effects of chemotherapy. However, in a report a pancreatic cancer trial in Germany, where the vector was introduced intra-arterially, several patients experienced mild disseminated intravascular coagulation [368768]. Additionally, there is concern about safety following the death of a patient from the University of Pennsylvania study of Ad vector containing a gene intended to correct OTCD. Since Sch-58500 conceptually uses the same type of vector, the FDA has asked Schering-Plough to discontinue enrolling patients in several studies until the reason for this death is determined.

Current Opinion

The use of Ad gene delivery vector represents an exciting approach to eliminating a variety of tumors deficient in the *p53* apoptosis-inducing gene. The Canji/Schering-Plough Ad vector appears to be relatively free of side effects when administered alone, and in combination with existing chemotherapeutic agents it offers an enhanced, less toxic alternative to traditional chemotherapeutics. This evaluation, however, concludes with a note of caution. Because of the death of a patient who was administered a related Ad vector in an trial conducted at the University of Pennsylvania, further new trials of Ad-based therapeutic agents have been temporarily suspended at the request of the FDA [343174]. These trials remain on hold, pending demonstration that an appropriate oversight and clinical monitoring program is in place [380063].

Licensing

Genzyme Molecular Oncology

In January 1998, Schering-Plough entered into a research collaboration with Genzyme Molecular Oncology (GMO) to develop gene therapies using GMO's lipid delivery system. The first year of the collaboration focused on the development of a delivery system for the gene p53. Schering-Plough will subsequently have the option of exclusive license to the technology for other, as yet undisclosed, gene therapies [273382].

Schering-Plough Corp

In October 1994, Schering-Plough formed an alliance with Canji to develop new cancer treatments based on Canji's p53 gene therapy technology. The agreement grants affiliated companies of Schering-Plough exclusive worldwide licenses to make, use and sell p53 tumor-suppressor gene products for human and animal uses. Under the agreement, Schering-Plough made an initial cash investment in Canji and was to make annual, performance and milestone payments over the next several years [168987]. Canji was acquired by Schering-Plough in 1996 [197761].

Development History

DEVELOPER	COUNTRY	STATUS	INDICATION	DATE	REFERENECE
Canji Inc	US	C3	Ovary tumor	23-JUN-99	323393
Canji Inc	US	C2	Lung tumor	05-JAN-98	273331
Canji Inc	US	C2	Colorectal tumor	12-MAY-99	324279
Schering-Plough Corp	US	C2	Lung tumor	01-JAN-98	273331
Schering-Plough Corp	US	C2	Colorectal tumor	12-MAY-99	324279
Canji Inc	US	C1	Liver tumor	01-JAN-96	237334
Canji Inc	US	C1	Melanoma	01-JAN-96	237334

Development Hist ry (continued)

DEVELOPER	COUNTRY	STATUS	INDICATION	DATE	REFERENECE
Canji Inc	US	C1	Neoplasm	01-JAN-96	237334
Canji Inc	US	C1	Breast tumor	01-JAN-96	237334
Canji Inc	US	C1	Head and neck tumor	01-JAN-96	237334
Schering-Plough Corp	US	C1	Melanoma	01-JAN-96	237334
Schering-Plough Corp	US	C1	Breast tumor	01-JAN-96	237334
Schering-Plough Corp	US	C1	Head and neck tumor	01-JAN-96	237334
Schering-Plough AB	US	C1	Liver tumor	01-JAN-96	237334
Schering-Plough AB	US	C1	Ovary tumor	01-DEC-98	315026
Schering-Plough Corp	US	DR	Leukemia	01-JAN-95	182509
Genzyme Molecular Oncology	US	DR	Neoplasm	17-MAR-98	273382
Canji Inc	US	DR	Leukemia	01-JAN-95	182509

Literature Classifications

Key references relating to the technology and are classified according to a set of standard headings to provide a quick guide to the bibliography. These headings are as follows:

Chemistry. References which discuss synthesis, construction and structure-activity relationships.

Biology: References which disclose aspects of the drugs pharmacology in animals

Metabolism: References which discuss metabolism, pharmacokinetics and toxicity.

Clinical: Reports of clinical phase studies in volunteers providing, where available, data on the following: whether the experiment is placebo-controlled or double- or single blind; number of patients; dosage.

Chemistry

STUDY TYPE	RESULTS	REFERENCE
Construction.	Successful construction of the rAd/p53 vector expressing p53 in a dose-dependent manner in cancer cells in culture.	168287
Purification.	Column chromatography purification of rAd/p53 vector expressing p53.	246629

Biology

STUDY TYPE	EFFECT STUDIED	EXPERIMENTAL MODEL	RESULTS	REFERENCE
In vitro	Transduction of tumor cells.	Cell culture.	Successful transduction of tumor cells with rAd/p53.	246630
In vivo	Expression.	Nude mice.	Successful ex vivo treatment of Saos-2 tumor cells followed by injection into nude mice resulted in complete tumor suppression using the rAd/p53 vector.	168287
In vivo	Expression.	Mouse xenografts.	Induction of apoptosis and growth reduction using the rAd/p53 vector to treat against 231 and 468 tumor xenografts.	246630
In vivo	Antitumor effect.	SCID-beige mice.	Reduction of the metastases of lung. Reduction of tumors in mice.	255115
In vivo	Penetrability.	Mouse tumor xenografts.	Depth of penetration of Sch-58500 determined in tumor tissue.	380042
In vivo	Dosage.	Human tumors.	Effective dosage range determined.	308167

Biology (c ntinued)

STUDY TYPE	EFFECT STUDIED	EXPERIMENTAL MODEL	RESULTS	REFERENCE
In vitro	Synergy.	Cultured tumor cells.	Enhanced sensitivity of tumor cells to anticancer agents when treated in combination with Sch-58500.	380035
In vivo	Tissue penetration/ permeability.	Human tumors.	Enhanced permeability of tumors to Sch-58500 when used in combination with paclitaxel.	284304

Metabolism

STUDY TYPE	EFFECT STUDIED	EXPERIMENTAL MODEL	RESULTS	REFERENCE
In vivo	Tissue distribution.	Human tumor biopsies.	Expression of p53 in p53-negative tumors from biopsies of patients treated with rAd/p53.	284932
In vivo	Tissue distribution.	Human tumor biopsies.	Immunohistochemical evidence of p53 expression in tumors and efficacy, despite antibody formation to the Ad5 vector.	339443

Clinical

EFFECT STUDIED	EXPERIMENTAL MODEL	RESULTS	REFERENCE
Phase III. Safety/biological activity.	Head and neck cancer trial ongoing, but no grade 3/4 toxicities reported.	Functional p53 DNA was detected in tumors by RT-PCR in four of ten patients whose results were available.	339443
Phase I/II. Safety/biological activity.	Variety of human tumors.	Transgene expression was detected by RT-PCR in many tumors of patients despite an antibody response to the adenovirus vector.	284304

Associated Patent

Title Gene therapy using replication competent targeted adenoviral vectors.

Assignee Canji Inc

Priority US-08433798 3-MAY-95

Publication WO-09634969 7-NOV-96

Inventors Gregory RJ, Huang W-M

Abstract

A novel method of treating cancer is claimed. The method involves the use of a replication-competent targeted Ad vector. The vector preferentially replicates in tumor cells due to activation of a tumor-specific gene regulatory region. These vectors can be used as a form of gene therapy to deliver therapeutic genes to treat cancer. Ad vectors were constructed using standard techniques to place the *E1a* gene under the control of a tumor-specific promoter, AFP. Therapeutic genes, such as a cytotoxic gene, were inserted into the Ad E3 region. The Ad vectors were assessed for their replication potential in tumor cell lines that can and cannot utilize the AFP promoter. The vectors of this invention were at a replication disadvantage compared to wild-type Ad in the AFP-negative cell line, HLF. However, they were able to replicate more efficiently in AFP-positive tumor cell lines.

References

• of special interest

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• *A summary of the testimony of William J Raub, Deputy Assistant Secretary for Science Policy Department of Health and Human Services, gave before the Subcommittee on Public Health Committee on Health, Education, Labor, And Pensions, explaining current and future precautions to be taken by the FDA and NIH for gene therapy trials*

Adenovirus-mediat d p53 gene therapy: Overview of preclinical studies and potential clinical applications

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Disruption of p53 function through mutation, or other means, occurs very frequently in human cancer and is associated with an unfavorable prognosis in various cancers. Evidence from in vitro and in vivo transduction experiments have demonstrated that adenoviral-mediated expression of wild-type p53 suppresses the transformed phenotype of many cell types and potentiates the cytotoxicity of both chemotherapeutic agents and radiation therapy. Recently several phase I studies have evaluated the safety, biological effect and different routes of administration of adenoviral-mediated p53 gene therapy in various tumor types. These studies indicate that adenovirus-mediated p53 gene therapy and introduction of wild-type p53 into tumor cells represents a potentially valuable tool for the therapy of many types of human cancers. This review presents an overview of the most recent advances in the preclinical and clinical evaluation of adenoviral p53 gene therapy as well as the challenges that lay ahead for future clinical studies.

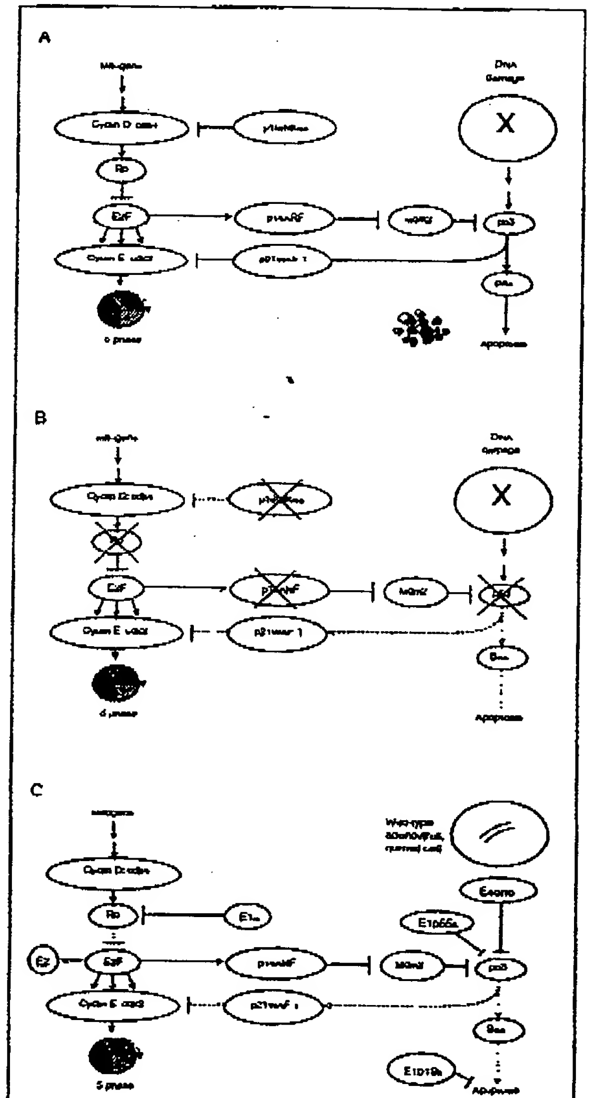
Introduction

Loss of p53 function appears to play a central role in a common pathway required for the development of most human cancers. p53 Mutations have been reported in nearly all tumor types and functional inactivation of p53 occurs in more than half of all cancers [1]. Although p53 is not required for normal development, patients with inherited p53 mutations (ie, Li-Fraumeni syndrome) and mice lacking one or both alleles of *p53* develop spontaneous tumors [2,3].

Inactivation of wild-type p53 can result from direct genetic mutations in the *p53* gene, binding of p53 protein by viral oncoproteins or cellular factors, or alteration of subcellular localization of p53 protein [4-8]. The inability of the cell to repair DNA damage leads to the accumulation of genetic changes that alter cellular responses to growth control. This has significant impact on the metastatic potential [9], as well as the response of tumor cells to therapeutic intervention [10,11]. In particular, the loss of p53 function has been associated with an unfavorable prognosis for cancers of the lung and breast, among others [12-14].

The *p53* tumor suppressor gene encodes a 393 amino acid nuclear phosphoprotein that plays a pivotal role in coordinating cellular responses to DNA damage and other forms of genotoxic stress. The p53 protein, a sequence-specific DNA transcription factor induces or represses the expression of multiple genes involved in regulating the cell cycle, DNA repair and poptosis [6-8]. Activation of wild-type p53 in response to DNA damage either causes cell cycle arrest or induces apoptosis. While the cyclin-dependent kinase inhibitor p21WAF1/CIP1 mediates p53-induced cell cycle arrest, the induction of apoptosis can involve transcription-dependent (Bax, Fas) and/or independent signaling pathways (Figure 1) [15]. Although the exact signal

Figure 1. Signaling pathways activated by DNA damage to wild-type *p53*.



Signal pathways leading from p53 to cell growth arrest or apoptosis. (A) Signal pathway in normal cells. (B) Cancer cells have defects in the Rb and p53 pathway. (C) Wild-type adenovirus protein interference with the Rb and p53 pathways in infected normal cells. *(Cancer J Sci Am Vol 5, 1999 p139-144, Copyright 1999 American Cancer Society. Reprinted by permission of Wiley-Liss Inc, a subsidiary of John Wiley & Sons Inc.)*

pathways leading from p53 to cell growth arrest or apoptosis are not fully understood, they are clearly regulated in a tissue-specific manner [16].

The *p53* gene has become a target for the development of new therapeutic strategies for cancer. One approach currently under clinical investigation is the introduction of wild-type *p53* tumor suppressor gene into tumor cells to achieve tumor suppression. Preclinical studies have confirmed that the introduction of wild-type p53 into neoplastic cells results in growth suppression and reduction of colony formation in soft agar [17]. Studies in nude mice indicate that introduction of p53 into tumor cells reduces their tumorigenicity [17], induces apoptosis in tumor xenograft models [18], increases sensitivity to several chemotherapeutic agents [19] and inhibits angiogenesis [20].

Considerable effort has been expended to design an effective method for *p53* gene therapy using several different viral and non-viral gene delivery systems [21-23]. Selection of a delivery system for introduction of *p53* is important for efficient transduction and sufficient expression of functional p53 protein *in vivo*. The ideal vector for gene therapy would be available at high titers, be easily reproducible, and elicit little to no immune response. Early studies using retrovirus-mediated gene transfer of wild-type *p53* into both human lung cell lines and xenograft models demonstrated that expression of wild-type p53 could lead to inhibition of tumor cell growth. However, poor stability of these vectors and the inability to produce high titers of highly infective recombinant virus have limited the use of retroviruses as a gene delivery system for *p53* gene therapy [23]. More recent studies have focused on the use of adenoviruses and other non-viral gene delivery systems. The type 5 adenoviral vector is currently the vector of choice for *in vitro* and *in vivo* studies due to its ability to transduce both proliferating and quiescent cells, ease of manipulation, and ability to produce high titers of highly infective recombinant virus. Additionally, the wild-type adenovirus is associated with minimal toxicity in humans.

The use of adenovirus-mediated gene therapy to introduce the *p53* gene into tumor cells is an evolving and potentially valuable approach to the treatment of many types of cancers currently resistant to therapeutic intervention. The main purpose of this review is to outline the most recent advances in the preclinical studies, clinical development of adenovirus-mediated *p53* gene therapy, and the challenges that lie ahead for future clinical studies.

Preclinical studies

The efficacy of adenovirus-mediated *p53* gene therapy has been demonstrated in numerous human cancer cell lines and xenograft models including those derived from lung, head and neck, breast, ovary, pancreas, prostate, brain and colorectal cancers [22]. Initial studies using recombinant human adenovirus-containing wild-type *p53* gene under the control of either the Ad2 major late promoter of human cytomegalovirus or the immediate early gene promoter demonstrated that introduction of the wild-type *p53* gene into tumor cells via these recombinant adenoviruses inhibited DNA synthesis in a p53-specific, dose-dependent manner [21]. In this study, injection of recombinant adenovirus encoding the wild-type *p53* gene into the peritumoral space of tumors derived from the $p53^{null}$ NIH-H69 human small-cell lung cancer cell line reduced tumor growth and increased survival time compared with controls. The effect of endogenous mutant *p53* on the ability of wild-type *p53* to suppress tumor cell growth was also assessed in a series of 45 human cell lines that contained either wild-type or mutated p53 protein or no p53 protein [24]. A positive correlation was observed between the percentage of tumor cells that were transduced and the antiproliferative effects of adenoviral *p53* in $p53^{null}$ and $p53^{mut}$ cells. However, infection with adenoviral *p53* had minimal effect on cells expressing wild-type p53. In human xenograft models, adenoviral *p53* gene transfer suppressed tumor growth in $p53^{null}$ and $p53^{mut}$ tumors, including tumors with dominant negative *p53* mutations, and increased survival times

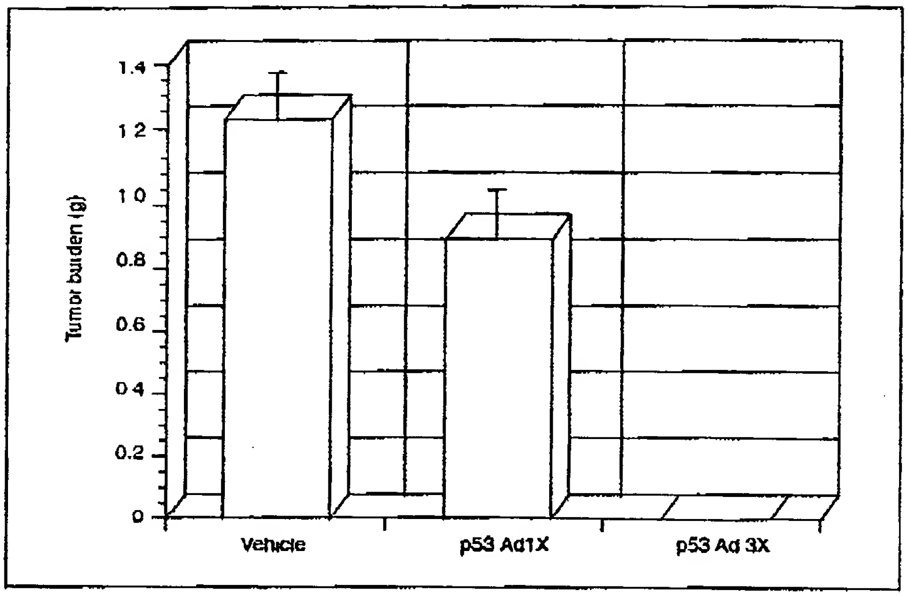
Recently the combination of adenoviral *p53* gene transfer with other modalities and in the sensitization of chemotherapy-resistant disease has been evaluated in a variety of tumor types. A brief review of the results from some of these studies and their clinical applications follows

Ovarian cancer

Based on the regional nature of the disease and antitumor activity in ovarian xenograft models, adenovirus-mediated *p53* gene therapy is currently being investigated in clinical trials for the treatment of recurrent ovarian cancer [21,25-31]. Mujoo *et al* [28] demonstrated the efficacy of adenovirus-mediated *p53* gene therapy in a highly aggressive ovarian SK-OV-3 xenograft model. In this study, *ex vivo* treatment of SK-OV-3 cells with recombinant adenoviral *p53* prior to infection into nude mice increased survival by more than 50% over control animals. Long-term survival of 166 to 423 days was noted in an intraperitoneal SK-OV-3 xenograft model treated with recombinant adenoviral *p53* [28]. Nielson *et al* [30] observed similar results in a study evaluating the efficacy of different dosing strategies in the SK-OV-3 xenograft model. Tumor burden was significantly reduced in all mice treated with adenoviral *p53* gene therapy ($P \leq 0.008$). In this study, fractionated doses of adenoviral *p53* had somewhat greater efficacy compared with a single bolus injection (Figure 2) [30]. In contrast, no survival advantag was observed for adenovirus-mediated *p53* gene therapy in human 2774 ovarian xenograft model [31]. It is possible that the type of *p53* mutations and presence of mismatch repair defect in the 2774 cell line may have contributed to the lack of p53-specific response observed *in vivo*.

The antitumor effect of *p53* gene therapy was also observed in human ovarian cancer cells that were resistant to cisplatin [27]. In this study, infection with adenoviral *p53* resulted in a 10-fold increase in sensitivity to cisplatin in the cisplatin-resistant C-1 cell line. Cell cycle analysis revealed that infection with adenoviral *p53* increased the number of cells undergoing apoptosis in cisplatin-resistant cells in comparison with parental cell line. Additionally, in an intraperitoneal C-1 xenograft model, *p53* gene therapy increased survival in more than 50% of the animals, demonstrating that *p53* adenoviral gene therapy may be useful in the treatment of drug-resistant disease. These results have lead to the investigation of intraperitoneal adenoviral *p53* gene therapy for the treatment of platinum and paclitaxel-resistant ovarian cancer.

Figure 2. Efficacy of fractional doses versus single b lus d ses of *p53*.



Fractionated dosing versus single bolus injection of adenoviral *p53* in SK-OV-3 ovarian xenograft model. *(Hum Gene Ther Vol 9 No 5, 1998 p681-694. Copyright 1998 Reprinted by permission of Mary Ann Liebert Inc.)*

Pancreatic cancer

Two recent studies revealed the antitumorigenic effects of adenovirus-mediated *p53* gene therapy in human pancreatic cancer both *in vitro* and *in vivo* [32,33]. Although transduction efficiency varied among the cell lines tested, adenovirus-mediated *p53* gene transfer suppressed growth of all human pancreatic tumor cell lines in a dose-dependent manner. A 4-fold increase in apoptotic cells was observed in MiaPaCa-2 cell line at 48 and 72 h following infection [32]. Similar effects were observed in xenografts established from these cell lines after intratumoral injections of adenoviral *p53*.

Bladder cancer

The application of *p53* gene therapy as an alternative treatment for bladder cancer has recently been investigated in an orthotopic model of bladder cancer in rats [34]. In this model, intravesicular administration of adenoviral *p53* resulted in increased p53 expression that corresponded with areas of apoptotic cell death in tumor tissues. Based on preclinical data, there are phase I clinical investigations evaluating intravesicular administration of adenoviral *p53* for the treatment of locally advanced bladder cancer in progress.

Hepatocellular carcinoma and intra-arterial delivery

The potential for intra-arterial delivery of adenovirus-mediated *p53* gene therapy for the treatment of liver malignancies has also been evaluated in a syngenic rat model of hepatocellular carcinoma [35]. For these studies, multifocal tumor nodules were produced in buffalo rats using the McA-RH7777 $p53^{mut}$ hepatocellular cell line. Intrahepatic arterial delivery of adenoviral *p53* increased expression of wild-type p53 and suppressed tumors when compared with untreated or mock-infected animals. Additionally, intrahepatic arterial dosing with adenoviral *p53* decreased systemic exposure to adenovirus compared with intravenous dosing. Based on these results, a phase I dose-escalation study has been initiated to evaluate the safety and potential gene transfer for intra-arterial administration of adenoviral *p53* in patients with colorectal liver metastasis.

Glioblastoma

Mutations and aberrations in the expression of the *p53* gene occur in 30% to 65% of all malignant gliomas, suggesting an early role in the initiation of tumorigenesis. Introduction of *p53* gene into glioma cell lines has been shown to induce apoptosis in tumor cells encoding mutant *p53* gene [36,37]. In these studies, introduction of adenoviral *p53* had a minimal effect on suppressing the growth of glioma cell lines encoding wild-type *p53* gene. However, the results of several recent studies that have investigated the combination of adenovirus-mediated *p53* gene therapy with ionizing radiation indicate there may be a role for *p53* gene therapy as an adjunct to radiation in the treatment of malignant glioma [38-40]. (See 'combination therapy' section.)

Head and neck squamous cell carcinoma

Several studies have demonstrated the antitumor effect of adenovirus-mediated *p53* gene therapy in human head and neck squamous cell carcinoma (HNSCC) cell lines and xenograft models [41-43]. Adenoviral *p53* induced growth arrest and morphological changes consistent with apoptosis in the Tu-138 HNSC cell line and xenograft model [41,42]. In additional studies using a subcutaneous microscopic

residual HNSCC xenograft model that mimics the post-surgical environment of head and neck cancer patients with advanced disease, adenovirus-mediated *p53* gene therapy suppressed tumors, regardless of the *p53* genotype of the tumor cells [43]. These results have led to the initiation of two phase I studies to determine the feasibility and safety of *p53* gene therapy in patients with advanced recurrent HNSCC.

Applications in chemotherapy-resistant breast cancer

Seth *et al* [44] evaluated the cytotoxic effects of adenovirus-mediated *p53* gene therapy in two breast cancer MCF-7 cell lines selected for resistance to adriamycin (MCF-Adr) and mitoxantrone (MCF-Mito). In this study, both MCF-Adr and MCF-Mito cell lines were 20- to 30-times more sensitive to the cytotoxic effects of adenoviral *p53* than parental MCF-7 cell lines. Infection with 3.2 pfu/cell of adenoviral *p53* resulted in a 2-fold reduction in the IC50 of adriamycin in adriamycin-resistant cells. Adenoviral *p53* infection induced apoptosis in both MCF-Adr and MCF-Mito cell lines while parental MCF-7 cell lines failed to undergo apoptosis. Additionally, infection of a mixed population of MCF-Adr and CD34+ cells with adenoviral *p53* selectively inhibited the growth of drug-resistant breast cancer cells and had no effect on CFU-GM colony formation from the CD34+ cells. These data suggest gene therapy may be effective in sensitizing cells to the effects of chemotherapy and also support a role for *p53* gene transfer in purging stem cell products of patients undergoing autologous bone marrow transplantation.

Combination of traditional therapy with adenovirus-mediated *p53* gene therapy

Drug resistance that develops in many different human cancers during initial therapy or relapse has substantial impact on the overall outcome and success of cancer therapy. The loss of functional p53 in different types of tumor cells has been associated with resistance to chemotherapeutic agents [45,46]. The efficacy of combining adenovirus-mediated *p53* gene therapy with chemotherapy has been investigated in a variety of different tumor types, including carcinomas of the lung, ovary, breast and colon [26,29,38,39,47-50].

Combination with cisplatin

Adenovirus-mediated *p53* gene therapy has been demonstrated to increase the sensitivity of a number of different tumor types to the cytotoxic effects of cisplatin. Fujiwara *et al* [19] were among the first to demonstrate that this combination had an additive effect on growth inhibition of the human lung cancer H358 cell line. Nguyen *et al* [51] reported similar results *in vitro* and *in vivo* using the $p53^{null}$ human lung cancer H1299 xenograft model where treatment of H1299 cells with low concentrations of cisplatin 48 h before infection with adenoviral *p53* inhibited growth 31% to 60%. A higher level of p53 protein expression and fraction of apoptotic cells was observed in cells treated with this combination compared with cells infected with adenoviral *p53* alone. Systemic administration of cisplatin before, during, or after the intratumoral administration of adenoviral *p53* also resulted in pronounced inhibition of tumor growth in the H1299 xenograft model. The administration of cisplatin before infection with adenoviral *p53* was the most effective *in vivo* dosing schedule. Additionally, a second cycle of gene therapy resulted in greater growth suppression compared with a single cycle of therapy [51]. This combination has also been used for the treatment of non-small-cell lung cancer (NSCLC) [52].

Similarly, Ogawa *et al* [49] demonstrated increased sensitivity to cisplatin cytotoxicity in the $p53^{mut}$ WiDr human colon cancer cell line and xenograft model transduced with adenoviral *p53*. Transduction of WiDr cells with 50 pfu/cell resulted in a high level of p53 expression with no cytotoxic effects. Combination with cisplatin produced an enhanced antitumor effect with highest growth suppression observed at 1 µg/ml of cisplatin. Administration of intraperitoneal cisplatin after intratumoral adenoviral *p53* significantly enhanced growth suppression in WiDr xenografts compared with adenoviral *p53* alone ($P < 0.05$). Kanamori *et al* [50] also noted significant growth suppression of SK-OV-3 cells treated with adenoviral *p53* gene therapy and cisplatin. In this study there was a positive correlation between level of adenoviral *p53* transduction and increased sensitivity of SK-OV-3 cells to cisplatin. Miyake *et al* [53] also noted increased sensitivity of a subcutaneous HT1376 human bladder xenograft model to cisplatin following introduction of adenoviral *p53*. Direct injection of adenoviral *p53* vector into pre-existing tumors, followed by intraperitoneal administration of cisplatin, induced apoptotic destruction of tumors. These findings suggest that the combination of adenovirus-mediated *p53* gene therapy and cisplatin may be an efficient tool for the treatment of cancer.

Combination with paclitaxel

Recently, Nielsen *et al* [29] demonstrated that combination of adenovirus-mediated *p53* gene therapy with paclitaxel increased the sensitivity of human head and neck, ovarian, prostate and breast cancer to the cytotoxic effects of paclitaxel *in vitro* and *in vivo*. In this study, pretreatment of cells with paclitaxel 24 h before exposure to adenoviral *p53* or with both agents simultaneously had either a synergistic or additive effect, depending on the cell line tested. Of interest was the observation that concentrations of paclitaxel, which were lower than that required for microtubule concentration, resulted in a dose-dependent increase in transduction of cells with adenoviral p53 vector. Cell cycle analysis revealed that cellular response to the combination depended on the relative concentrations of the two agents. Higher levels of paclitaxel yielded G2 arrest, while higher levels of adenoviral *p53* resulted in a G0/G1 arrest prior to apoptosis. *In vivo*, combination of paclitaxel and adenoviral *p53* gene therapy produced significant reduction in tumor growth in ovarian (SK-OV-3), prostate (DU-145) and two breast (MDA-MB 468 and MDA-MB 231) xenograft models compared with either treatment alone. These data indicate that combination of paclitaxel and adenoviral *p53* gene therapy is effective in different tumor types.

Combination with IL-2

Putzer *et al* [48] evaluated the efficacy of combined adenoviral gene therapy with p53 and IL-2 expressing vectors to stimulate immune specific antitumor response and tumor regression in a transgenic breast xenograft model. Single intratumoral injection of adenoviral *p53* (1×10^9 pfu) and low doses of adenoviral *IL-2* (1.5×10^8 pfu) resulted in 65% reduction in tumor size without toxicity. In

contrast, treatment with either vector alone at the same dose resulted in delayed tumor growth. Tumor regression was associated with long-term immunity, since 50% of mice remained tumor free and were immune to rechallenge with fresh tumor cells. Combination therapy was also associated with development of specific cytolytic T-lymphocyte response compared with either treatment alone.

Combination with 2-methoxyestradiol

Kataoka *et al* [54] evaluated the combination of adenovirus-mediated *p53* gene therapy with 2-methoxyestradiol in human metastatic lung cancer cells *in vivo* as a method for improving the effectiveness of *p53* gene therapy in the treatment of lung metastases. Simultaneous administration of *p53* and 2-methoxyestradiol resulted in a greater than additive reduction, with the lung colony count reduced by 33% compared with control values. These results suggest that the synergistic effect of this combination may have an application in the systemic treatment of lung cancer

Combination with irradiation

The effect of adenovirus-mediated *p53* gene therapy on the radiosensitivity of tumor cells has been the focus of several recent studies [26,38,39,55-57]. Spitz *et al* [55] examined the effect of adenoviral *p53* gene therapy and irradiation on p53^{mut} SW620 colorectal tumor cells *in vitro* and *in vivo*. Transduction of cells with adenoviral *p53* 2 days prior to irradiation with 2 Gy resulted in 50% to 60% reduction in cell survival via apoptosis compared with cells that were mock- or vector-infected prior to irradiation. This combination also produced significant tumor growth suppression in subcutaneous SW620 xenografts pretreated with three consecutive doses of adenoviral *p53* prior to 5 Gy of irradiation ($P < 0.01$). Similar results were observed in a p53$^{-/-}$ SK-OV-3 ovarian xenograft model [26]. In this study, intratumoral administration of adenoviral *p53* (10^9 pfu) 2 days before treatment with radiation led to a 45% reduction in tumor size compared with either treatment alone, in mock-infected and untreated controls.

The ability of adenovirus-mediated *p53* gene therapy to sensitize human glioma cells that encode mutant p53 to irradiation has also been evaluated. Introduction of wild-type *p53* into the p53^{mt} human U87MG glioma cell line via adenoviral vector 2 days before exposure to irradiation (9 Gy dose) significantly increased radiation-induced apoptosis compared with mock-infected controls ($P < 0.001$) [38]. Further analysis showed that irradiation of U87MG glioma cells infected with adenoviral *p53* resulted in increased expression of both p53 protein and p21 mRNA levels.

Badie *et al* [39] also investigated the combination of adenovirus-mediated *p53* gene therapy and irradiation in a rat 9L gliosarcoma xenograft model. Stereotactic injection of adenoviral *p53* (10^9 pfu/ml) into pre-existing brain tumors resulted in a modest reduction in tumor volume However, administration before radiation produced a significant (85%) reduction in tumor size compared with control animals ($P < 0.0008$). Moreover, combination therapy improved survival, with 29% (2/7) of animals in the combined treatment group remaining tumor free 2 weeks after treatment. Analysis of brain tissue from surviving animals in the combined treatment group revealed no microscopic evidence of tumor. Although these results have important implications for improving the treatment of malignant glioma and metastatic brain tumors, further studies in human brain tumor xenograft models with different p53 status are needed to confirm the efficacy of this combination *in vivo*.

p53-Mediated sensitization of HNSCC cells to radiotherapy has also been demonstrated *in vitro* and *in vivo* [56,57]. Treatment of radiation resistant JSQ-3 HNSCC cell line with adenoviral *p53* inhibited growth *in vitro* and *in vivo* while having no effect on normal cells. More significantly, introduction of *p53* also resulted in a dose-dependent reduction in the radiation resistance. A single dose of adenoviral *p53* combined with ionizing radiation markedly enhanced radiosensitivity of JSQ-3 xenograft with complete long-term regression of tumors for up to 162 days. These results provide further evidence of the efficacy of this combination and indicate that adenoviral *p53* sensitization of tumors to radiation therapy may significantly reduce the rate of recurrence of certain tumors after radiation treatment.

Clinical studies of adenovirus-mediated *p53* gene therapy

Extensive preclinical studies have evaluated the safety of using replication-deficient type 5 adenoviral vectors encoding wild-type *p53* under the control of the human cytomegalovirus immediate early gene promoter to transfer genes to human cells [21,24]. In these studies, doses of the adenoviral *p53* that are cytotoxic to neoplastic cells had little to no adverse effect on normal cells including fibroblasts, bone marrow cells, and epithelium from the liver, lungs, breast and ovary [41,58,59]. These studies have also demonstrated that intratumoral, intrahepatic and intraperitoneal routes of administration with adenoviral *p53* do not adversely affect surrounding tissues. Similar results have been reported for phase I studies that evaluated the safety and biological effect of adenovirus-mediated *p53* gene therapy in the treatment of primary and metastatic head and neck, lung, liver, colorectal and ovarian tumors (Table 1) [52,60,62-64]. The results of these studies, just now beginning to appear, are summarized below.

Intratumoral delivery

Non-small-cell lung cancer

A phase I single-dose rising study has evaluated adenovirus-mediated *p53* gene transfer in advanced NSCLC [52]. Tumors from 15 patients with incurable NSCLC were transduced with one of four doses of single agent adenoviral *p53* ranging from 10^7 to 10^{10} pfu/ml. Adenoviral *p53* was administered as a single bronchoscopic or computed tomography (CT)-guided percutaneous intratumoral injection. The tumors from all patients had high levels of p53 as detected by immunohistochemistry, suggesting mutations in the *p53* gene. Successful gene transfer and expression of exogenous wild-type p53 occurred at higher concentrations of adenoviral *p53* (10^9 pfu/ml) and vector-related sequences were detected in post-treatment biopsies from six patients. Stabilization of tumor growth was achieved in four of these patients and no clinically significant toxicity due to *p53* therapy was observed [52].

Table 1. Completed clinical trials of adenovirus-mediated *p53* gene therapy.

Disease	Adenoviral p53 dose	Response	Reference
NSCLC	10^7 to 10^{10} pfu/ml	Stable disease was achieved in 4/15	[52]
NSCLC	10^6 to 10^{11} pfu with or without iv cisplatin	10% partial response, 61% stable disease, 25% progressive disease, 74% stable disease for CDDP+ ad*p53*	[60]
Advanced recurrent HNSCC	10^6 to 10^{12} pfu	N/A	[62]
HNSCC	10^6 to 10^{11} pfu over 2 weeks to 6.5 months	18% stable disease, 6% partial response, 3% complete response	[63]
Ovarian	7.5×10^{10} to 7.5×10^{12} particles	N/A	[64]

NSCLC = non-small-cell lung cancer, HNSCC = human head and neck squamous cell carcinoma, CDDP = cisplatin (cis-diamminedichloroplatinum(II)).

Additionally, the safety and therapeutic potential of adenoviral *p53* gene therapy with or without cisplatin in patients with advanced NSCLC who failed conventional therapy was evaluated [60,61]. In this study, 28 patients (89% before radiation and 75% before chemotherapy) were treated with or without intravenous cisplatin 3 days prior to bronchoscopic or CT-guided percutaneous intratumoral injection of escalating doses of adenoviral *p53* (10^6 to 10^{11} pfu) [61]. Patients received up to six intratumoral injections of adenoviral *p53* at monthly intervals. A total of 84 courses were administered, with 56 doses (67%) being repeat injections. The majority of patients (68%) received up to three courses of adenoviral *p53*, while 11%, 7% and 14% patients received four, five and six courses of adenoviral *p53*, respectively. Adenoviral *p53* was well-tolerated and produced little toxicity. Vector-related sequences were detected in post-treatment biopsies. Of the 25 patients evaluable for tumor response, two achieved a partial response, 16 demonstrated stable disease and seven progressed after treatment with adenoviral *p53* alone. Transient local control of disease ranged from 2 to 14 months, and more than 50% reduction in tumor size was observed in two patients who received six courses of adenoviral *p53* [61]. A cohort of nine additional patients received adenoviral *p53* in conjunction with cisplatin given at a dose of 80 mg/m^2 intravenously over 2 h, 3 days before injection with adenoviral *p53* [60]. Stabilization of disease was slightly higher (74% CDDP+ ad*p53* verses 61% for ad*p53* alone) in this cohort of patients compared with those who received adenoviral *p53* alone. An analysis of factors that affect disease progression revealed that higher doses of adenoviral *p53*, concomitant cisplatin therapy, and increased apoptosis as demonstrated by *in situ* DNA nick end labeling staining of tumor specimens were associated with enhanced time to progression [61]. These encouraging results have precipitated the design of a phase II study to assess the efficacy of adenovirus-mediated *p53* gene therapy in combination with radiation.

Advanced recurrent head and neck squamous cell carcinoma

Two phase I clinical studies have evaluated the safety and biological activity of adenovirus-mediated *p53* gene therapy in patients with resectable and non-resectable advanced recurrent HNSCC [62,63]. In one study, HNSCC tumors from 25 patients were transduced with adenoviral *p53* at doses ranging from 10^9 to 10^{12} pfu [62]. A singl injection of either 7.5×10^9 pfu, 7.5×10^{10} pfu or 7.5×10^{11} pfu was administered to three groups of three patients each, respectively. Multiple injections of either 7.5×10^{11} pfu or 1.5×10^{12} pfu were administered to six patients and 10 patients, respectively. Of the patients who received multiple injections, three patients at the 7.5×10^{11} pfu dosage level and six patients at 1.5×10^{12} pfu dosage level received chemotherapy concurrently. Successful transduction of tumor was observed in four of 10 tumors examined and response to therapy was observed in one patient [62].

Clayman *et al* [63] also evaluated the safety and therapeutic potential of adenovirus-mediated *p53* gene therapy in patients with resectable and non-resectable advanced recurrent HNSCC. In this study, 33 patients received multiple intratumoral doses of adenoviral *p53* alone ranging from 10^6 pfu to 10^{11} pfu over a course of 2 weeks to 6.5 months. In patients with non-resectable tumors, objective tumor regression of > 50% was observed in two patients, while stabilization of disease for up to 3.5 months was achieved in another six patients. Additionally, one patient with resectable disease was considered to have achieved a complete pathological response in that no viable tumor was found in the completely resected specimen [63]. Based on these results, a phase II study is currently examining the effect of adenoviral *p53* gene therapy on response rate, duration of response, time to progression, overall survival, and quality of life in patients with recurrent HNSCC.

Intraperitoneal delivery: Recurrent ovarian cancer

The efficacy of intraperitoneal *p53* gene therapy alone or in combination with chemotherapy was evaluated in 37 women with advanced ovarian cancer that was refractory to conventional therapy [64]. Patients received a single dose of adenoviral *p53* ranging from 7.5×10^{10} to 7.5×10^{12} particle number (pn). Expression of transgene was detected by reverse transcriptase polymerase chain reaction (RT-PCR) in some tumor samples at the lowest dose level and consistently at the 7.5×10^{11} pn dose level and above. Once the safety of single injection was established, patients received multiple daily doses of adenoviral *p53* ranging from 7.5×10^{10} to 7.5×10^{12} pn concurrently with chemotherapy every 21 to 28 days. The highest dose evaluated was 7.5×10^{12} pn daily for 5 days concurrently

with chemotherapy and was well-tolerated with mild analgesic and antipyretic prophylaxis [64]. The maximum tolerated dose for this study was not identified. Overall the intraperitoneal administration of adenoviral *p53* gene therapy was well-tolerated [64]. Further phase I studies continue to evaluate intraperitoneal *p53* gene therapy alone or in combination with other chemotherapeutic agents for the treatment of advanced, recurrent, or persistent ovarian cancer, as well as in the treatment of platinum- and paclitaxel-resistant ovarian cancer.

Intrahepatic arterial delivery: Colorectal liver metastasis and hepatocellular carcinoma

A phase I dose-escalation study has been initiated to evaluate the safety and gene transfer for intra-arterial administration of adenoviral *p53* in patients with colorectal liver metastasis and hepatocellular carcinoma [65]. Currently 16 patients with immunohistochemical evidence of *p53* mutation have been enrolled. Cohorts of three patients each have received doses of adenoviral *p53* beginning at 7.5×10^{9} pfu and escalating to 7.5×10^{13} pfu. The maximum tolerated dose was defined at 2.5×10^{13} pfu. Expression of transgene has been detected in the tumor by RT-PCR at the 2.5×10^{11} pfu dose level [65]. Intra-arterial administration of adenoviral *p53* gene therapy has been well-tolerated and evidence of dose-limiting toxicity has been observed at the highest dose level [Horowitz JA, unpublished data]. Evaluation of dose-escalation of adenoviral *p53* combined with chemotherapy continues.

The results of these phase I studies demonstrate that intratumoral, intraperitoneal and intrahepatic arterial delivery of adenoviral *p53* gene therapy is well-tolerated and results in the successful expression of wild-type *p53* into various tumor types. Anecdotal reports of clinical responses support further investigation. Several additional studies have been initiated to evaluate the use of adenovirus-mediated *p53* gene therapy in the treatment of other malignancies, including bladder cancer and malignant glioma (Table 2). Phase II studies have been initiated to further evaluate adenoviral *p53* gene therapy in the treatment of HNSCC, NSCLC, and ovarian and colorectal cancers.

Issues for adenovirus-mediated *p53* gene therapy

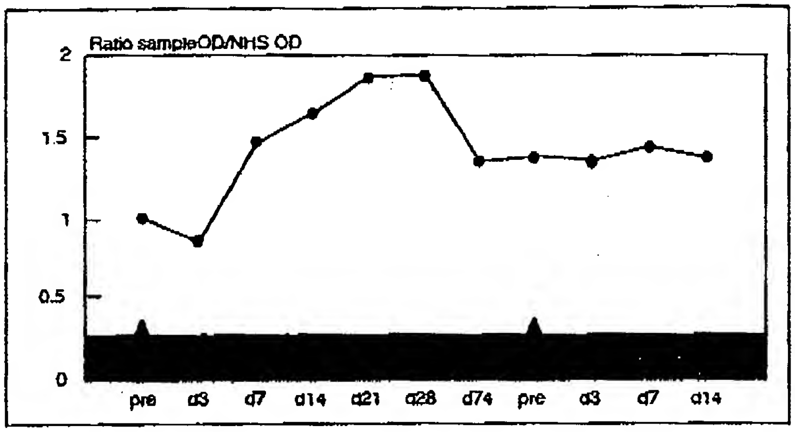
The above preclinical and clinical phase I studies confirm that the exogenous transgene can be expressed in tumors by various routes of administration, therefore confirming the proof of concept. The largest obstacle to human gene therapy is the delivery of the transgene to the tumor site. This issue affects all delivery systems identified to date. The issue specific to adenoviruses is the rapid clearance of the vector and induction of host immune response to the adenovirus. This may result in the requirement for higher doses of adenovirus to overcome this obstacle, or the need for the vector to be delivered by intratumoral or regional intrahepatic artery or intraperitoneal route.

Most advanced malignancies are systemic in nature, and delivery by intratumoral or regional routes place limitations on gene therapy. The development of alternative delivery systems, and means by which this delivery system can be administered systemically, are under investigation. Until such alternatives are available, the addition of systemic chemotherapy enhances this form of novel therapy for advanced disease. It is encouraging that the individual patient's pre-existing immunity to adenovirus has not precluded expression of the exogenous transgene (Figure 3) [52] and that the safety profile of these agents when combined with chemotherapy are acceptable. The impact that this immunity has on the dose intensity, however, can be inferred. In addition, it is encouraging that the wide tissue tropism of the adenoviruses has not resulted in undue or unmanageable safety issues, despite published preclinical models where hepatic toxicity was observed [30,66,67].

Table 2. Ongoing clinical trials of adenovirus-mediated *p53* gene therapy.

Phase	Description
Phase I dose-escalation study	Intravesicular administration of adenoviral *p53* for treatment of locally advanced and metastatic bladder cancer
Phase I multicenter dose-escalation study	Intratumoral stereotactic injection of adenoviral *p53* for treatment of recurrent malignant glioma
Phase I dose-escalation study	Intraperitoneal delivery of adenoviral p53 for treatment of advanced, recurrent, or persistent ovarian cancer
Phase I dose-escalation study	Intraperitoneal delivery of adenoviral *p53* for treatment of platinum- and paclitaxel-resistant ovarian cancer
Phase I pilot dose-escalation study	Delivery of adenoviral *p53* by bronchoalveolar lavage for treatment of bronchoalveolar cell lung cancer
Phase I dose-escalation study	Percutaneous injections of adenoviral *p53* for hepatocellular carcinoma
Phase I dose-escalation study	Intra-arterial delivery of adenoviral *p53* for treatment of primary and metastatic tumors of the liver
Phase I dose-escalation study	Combination of chemotherapy with single and multiple intraperitoneal injections adenoviral *p53* for treatment of peritoneal carcinomas
Phase II study	Recurrent squamous cell carcinoma of the head and neck
Phase II/III	Intraperitoneal delivery of *p53* adenovirus for treatment of newly diagnosed ovarian cancer
Phase II	Intra-arterial delivery of *p53* adenovirus for treatment of colon cancer metastatic to the liver

Figure 3. Development of neutralizing antibodies against adenoviral vector following the first treatment with aden viral *p53*.



Course of anti-adenoviral p53 antibodies in a single patient receiving two doses of adenoviral *p53*. The black triangles show the time points of treatment. The shaded area highlights the negative threshold of 0.28 (Hum Gene Ther Vol 9 No 14, 1998 p2675-2082 Copyright 1998 Reprinted by permission of Mary Ann Liebert Inc.)

Conclusion

The results of preclinical and clinical studies have demonstrated that adenovirus-mediated gene therapy is a safe and efficient method for the introduction of the wild-type *p53* gene in a variety of human cancers. It is clear that the treatment of tumor cells with adenoviral *p53* causes tumor regression. Evidence from *in vitro* and *in vivo* studies indicate that adenovirus-mediated *p53* gene therapy potentiates the cytotoxicity of both chemotherapeutic agents and radiation therapy in a variety of cancers. Results from initial clinical studies have confirmed the safety of adenovirus-mediated *p53* gene therapy. Future studies will be needed to determine the efficacy of adenovirus-mediated *p53* gene therapy and its role in the management of cancer patients. Such studies are underway.

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Successful Adenovirus-Mediated Wild-Type *p53* Gene Transfer in Patients With Bladder Cancer by Intravesical Vector Instillation

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Purpose: To study safety, feasibility, and biologic activity of adenovirus-mediated *p53* gene transfer in patients with bladder cancer.

Patients and Methods: Twelve patients with histologically confirmed bladder cancer scheduled for cystectomy were treated on day 1 with a single intratumoral injection of SCH 58500 (rAd/p53) at cystoscopy at one dose level (7.5×10^{11} particles) or a single intravesical instillation of SCH 58500 with a transduction-enhancing agent (Big CHAP) at three dose levels (7.5×10^{11} to 7.5×10^{13} particles). Cystectomies were performed in 11 patients on day 3, and transgene expression, vector distribution, and biologic markers of transgene activity were assessed by molecular and immunohistochemical methods in tumors and normal bladder samples.

Results: Specific transgene expression was detected in tissues from seven of eight assessable patients treated with intravesical instillation of SCH 58500 but in none of three assessable patients treated with intratumoral injection of SCH 58500. Induction of RNA and protein expression of the p53 target gene *p21/WAF1* was demonstrated in samples from patients treated with SCH 58500 instillation at higher dose levels. Distribution studies after intravesical instillation of SCH 58500 revealed both high transduction efficacy and vector penetration throughout the whole urothelium and into submucosal tumor cells. No dose-limiting toxicity was observed, and side effects were local and of transient nature.

Conclusion: Intravesical instillation of SCH 58500 combined with a transduction-enhancing agent is safe, feasible, and biologically active in patients with bladder cancer. Studies to evaluate the clinical efficacy of this treatment in patients with localized high-risk bladder cancer are warranted.

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AN ESTIMATED 261,000 new cases of bladder cancer are diagnosed worldwide per year. Bladder cancer is prevalent in the developed countries, where it affects mainly men and is frequently associated with a history of tobacco smoking or some occupational exposures, and in Northern Africa and Western Asia, where it is related to endemic infection with the parasite *Schistosoma mansonii*.[1] In the Western world, 70% to 80% of patients present with superficial bladder tumors, which can be treated with transurethral resection.[2,3] However, patients with less differentiated, large or multilocular bladder tumors as well as patients with carcinoma in situ or stage I bladder cancer are at high risk for tumor recurrence and development of muscle-invasive disease or distant metastases.[4,5] Treatment strategies for such high-risk patients include local resection with close surveillance,[2] local resection and intravesical therapy using bacillus Calmette-Guerin or cytotoxic agents,[6-8] or radical cystectomy with urinary diversion or reconstructive surgery.[9,10] Radical cystectomy provides optimal control of the bladder tumor, but at the price of organ loss. Intravesical and systemic medical therapies have substantial toxicities and bear the risk of local recurrence or tumor progression. Thus, new bladder-preserving treatment options for high-risk bladder cancer are required.

Mutations of the *p53* tumor suppressor gene are the most common genetic alteration in human cancers.[11] The role of p53 in the prevention of oncogenic transformation, maintenance of genetic stability, and sensitivity to commonly used cancer treatments is well established.[12,13] In some but not all studies, nuclear accumulation of p53 as an indicator for mutations in the p53 DNA binding domain was associated with an adverse prognosis in patients with bladder cancer.[14-17] Hence, somatic gene transfer of the *p53* tumor suppressor is an attractive new treatment modality for malignant bladder tumors. Preclinical cancer models have

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demonstrated that the expression of *p53* by viral or nonviral gene transfer technology effectively induced apoptosis or sensitized cancer cells to drug- or radiation-induced cell death.[18] These results have fostered the translation of *p53* gene therapy into early clinical studies, which were conducted in patients with advanced lung, head and neck, ovarian, or liver cancers.[19-23] Using intratumoral injection of adenoviral[20-22,24] or retroviral[19] *p53* expression vectors, local transgene expression[20,22] and evidence for local tumor regressions and induction of apoptosis[19,24] were reported from several phase I and pilot studies. However, the only controlled phase II study in patients with newly diagnosed advanced non–small-cell lung cancer (NSCLC) failed to demonstrate a significant clinical benefit from local *p53* gene transfer by intratumoral vector injection in combination with an effective first-line chemotherapy.[25] One reason for this apparent clinical inactivity might be insufficient gene delivery and transduction after intratumoral injection of adenoviral *p53* expression vectors. Systematic studies of these important parameters, however, are absent in cancer patients.

One way to overcome the potential limitations of the intratumoral injection approach is the instillation of high-vector doses into cavitary organs, such as the pleural space,[26] peritoneal cavity, or bladder. This should allow a homogeneous vector distribution along the tumor surfaces, as opposed to a vector distribution along the track of an injection needle. Preclinical studies have demonstrated the feasibility of this approach and have highlighted the importance of the addition of transduction-enhancing agents to maximize transgene expression in the bladder.[27,28]

To address this hypothesis, a study of safety, feasibility, and biologic activity of an intravesical instillation or an intratumoral injection of an adenoviral expression vector encoding wild-type p53 (SCH 58500) was conducted in patients with invasive bladder cancer. To allow assessment of vector distribution, transgene expression, and induction of p53 target genes or additional markers of biologic activity after the study treatment, only patients scheduled for radical cystectomy were enrolled onto this trial, enabling extensive tissue sampling for these analyses.

PATIENTS AND METHODS

Patients

Adult patients with histologically confirmed, muscle-invasive bladder cancer and indication for radical cystectomy were eligible for enrollment. Additional inclusion criteria were a life expectancy of at least 3 months, a Karnofsky performance score of at least 70%, and the absence of any clinical or laboratory evidence (WBC count ≥ 3,000/μL, absolute neutrophil count ≥ 1,000/μL, platelet count ≥ 100,000/μL, creatinine < 1.5 mg/dL, bilirubin < 1.5 mg/dL, AST and ALT < 1.5 times the upper limit of normal, and prothrombin and partial thromboplastin times within normal limits) for dysfunction of the hematopoietic, liver, renal, or coagulation systems. An interval of at least 4 weeks between prior chemotherapy, radiation, or major surgery was mandatory. Pregnant or nursing women, fertile women not practicing medically accepted contraception, patients with uncontrolled serious bacterial, fungal, or viral infections, human immunodeficiency virus–positive patients, and immunosuppressed patients were not eligible. Molecular or immunohistochemical evidence for an intratumoral *p53* mutation was not required for eligibility. All patients provided written informed consent. After written informed consent, control tissue samples were obtained from patients with advanced bladder cancer or patients with nonmalignant bladder disease treated by cystectomy.

Study Design

This was an open-label, single-center, phase I dose-escalation study of a single intratumoral injection (part A) or a single intravesical instillation (part B) of SCH 58500 (rAd/p53). Three patients were treated at each dose level, and dose escalation proceeded if no dose-limiting toxicity was observed. A dose-limiting toxicity was defined as any World Health Organization (WHO) grade 4 toxicity or any WHO grade 3 toxicity lasting more than 1 week. Adverse events that were clearly related to cystoscopy, catheter placement, cystectomy, or palliative treatment to the tumor were not considered dose-limiting. The protocol was approved by the local ethics committee (Bezirksärztekammer Rheinhessen) and the National Regulatory Office (Kommission Somatische Gentherapie der Bundesärztekammer). The study was conducted according to the Declaration of Helsinki (amended version, Hong Kong, 1989) and following the principles of good clinical practice.

Study Treatments

SCH 58500 is a replication-defective recombinant adenoviral vector encoding the complete human wild-type *p53* cDNA.[20,29] Doses were 7.5×10^{11} particles in level 1, 7.5×10^{12} particles in level 2, and 7.5×10^{13} particles in level 3. Patients treated in part A received a single intratumoral injection of 1 mL SCH 58500 in a standard saline-based solution[20] at cystoscopy on day 1. Patients treated in part B received a single intravesical instillation (total volume, 120 mL) of SCH 58500 in 20 mg/mL solution of Big CHAP, a transduction-enhancing agent,[28] through a transurethral catheter on day 1. After instillation, the catheters were blocked to allow a contact time of 60 minutes, followed by release of the catheter and extensive bladder irrigation with saline. During the course of the study, the vector instillation was divided into two sequential administrations of 50% of the vector dose each. The planned contact time for each half dose was 30 minutes; the second instillation immediately followed the release of the first dose. After treatment, all patients were hospitalized in single rooms in a biosafety environment at the study center for at least 24 hours or until adenovirus shedding was no longer detectable. Approximately 48 hours after vector administration (day 3), all patients underwent routinely scheduled radical cystectomies, which were not part of the study treatment.

Study End Points

The primary objective of this study was to assess the safety, feasibility, and toxicity of a single dose of SCH 58500 administered by intratumoral injection (part A) or by intravesical instillation (part B) in patients with invasive bladder cancer. Secondary end points were to

Table 1. Sequences of the Oligonucleotide Primers and Probes Used in Real-Time RT-PCR Assays

Target Gene	Function	Sequence	Expected PCR Product Size (bp)
p21/WAF1	Forward primer	TGGAGACTCTCAGGGTCGAAA	65
	Reverse primer	GGCGTTTGGAGTGGTAGAAATC	
	Probe	CGGCGGCAGACCAGCATGAC	
SCH 58500 DNA and RNA	Forward primer	AACGGTACTCCGCCACC	94
	Reverse primer	CGTGTCACCGTCGTGGA	
	Probe	CAGCTGCTCGAGAGGTTTTCCGATCC	
GAPDH	Forward primer	GAAGGTGAAGGTCGGAGTC	226
	Reverse primer	GAAGATGGTGATGGGATTTC	
	Probe	CAAGCTTCCCGTTCTCAGCC	

NOTE. All of the probes were labeled with the reporter signal FAM and TAMRA as the quencher.

assess vector distribution in normal and malignant bladder tissue, transgene expression, and markers of biologic activity in samples obtained at cystectomy.

Clinical Monitoring

Patients were closely monitored for adverse events for the first 7 days after study treatment. After hospital discharge, the patients were followed bimonthly for 1 year at the study center. The monitoring for the first 7 days after treatment included assessment of clinical symptoms, physical examination, monitoring of vital signs, Karnofsky index, concomitant medication, and recording of adverse events. Hematology, serum chemistry, and urinalysis were performed before treatment and on days 1, 2, 4, and 6 and during follow-up visits.

Virology Studies

Adenovirus shedding was monitored in urine, stool, or rectal swab specimens by means of a qualitative enzyme-linked immunosorbent assay (ELISA) before treatment on days 2 and 3 and until no adenovirus shedding was detectable.[20] In addition, urine samples were collected at multiple time points after study treatment and were examined for the presence of infectious adenoviruses by a flow cytometry–based infectivity assay.[30]

Detection of SCH 58500 DNA and Expression of Transgenic p53, p21/WAF1, *and the Coxsackie and Adenovirus Receptors*

SCH 58500 virus DNA, vector-specific transgene expression, p53 target gene *p21/WAF1* expression,[31,32] and Coxsackie and adenovirus receptor (CAR) expression were assessed in tumor samples and normal bladder tissue obtained at cystectomy by reverse transcriptase polymerase chain reaction (RT-PCR), as described previously,[20] and quantitative real-time PCR,[33,34] as described previously.[35] In brief, DNA and RNA were coextracted from frozen bladder samples using Trireagent (Molecular Research Center, Cincinnati, OH). Extracted RNA was DNased, and PCR was performed to ensure no DNA contamination. Real-time quantitative PCR and RT-PCR were performed using the ABI 7700 sequence detector (Applied Biosystems, Foster City, CA). The *GAPDH* gene was used as an internal control to assess the quality of assay samples. Gene expression results were expressed as number of copies per 1,000 copies of *GAPDH*. SCH 58500 DNA was quantified by comparison to viral DNA extracted from purified SCH 58500 virus (Qiagen, Valencia, CA). cRNAs were used as standards to quantify *p53, p21,* and *GAPDH* gene expression. The sequences of the oligonucleotide primers and probes are listed in Table 1. Primers for SCH 58500 gene and its expression were designed specifically to amplify SCH 58500 but not the human *p53* gene. Whenever possible, assays were performed on at least two different samples of tumor or nontumor tissue per patient. Bladder tissue samples obtained from patients with advanced bladder cancer, not treated with SCH 58500 served as negative controls. A cutoff level for positive real time PCR samples was set as the detection of at least 10 copies per reaction.[35]

Analysis of Tissue Sections

Localization of SCH 58500 was assessed using a direct in situ PCR method.[36] Formalin-fixed paraffin-embedded tissues were cut into 5-μm sections, placed on in situ PCR slides, and baked for 2 to 3 hours at 60°C on a slide hot plate. The slides were washed in xylene to remove the paraffin, followed by an incubation with 0.02 N HCl and digestion with 2.5 μg/mL proteinase K (Qiagen) at 37°C for 30 minutes. The endogenous alkaline phosphatase activity was eliminated by incubating the slides in ice-cold 20% (vol/vol) acetic acid. Slides were dehydrated in graded alcohols and rehydrated in 45 μL of PCR master mix containing 1 μmol/L of each dinitro-phenyl (DNP)-labeled primer, 200 μmol/L of each dNTP, 2.5 mmol/L magnesium chloride, and 10 units of AmpliTaq DNA polymerase (Applied Biosystems). Primers were designed to amplify a SCH 58500-specific sequence located between the cytomegalovirus promoter (5'-CGTGTCACCGTCGTGGA-3') and the upstream *p53* cDNA (5'-CCACTGCTTACTGGCTTATCGAAAT-3'). This primer selection prevents the amplification of genomic *p53* DNA.[29] Reactions were performed in a Perkin Elmer Gene Amp In Situ PCR System 1000 (Applied Biosystems) programmed for one cycle of denaturation at 95°C for 5 minutes and annealing at 55°C for 90 seconds, followed by 34 cycles of 94°C for 30 seconds and 55°C for 90 seconds. After completion of the PCR, slides were washed two times with standard saline citrate (0.3 mol/L NaCl and 0.03 mol/L sodium citrate) and blocked with casein solution (Vector, Burlingame, CA). For tissue sections, the DNP molecules incorporated into the PCR amplicons were detected using an anti-DNP antibody conjugated with alkaline phosphatase (Applied Biosystems). The sections were stained using the alkaline phosphatase substrate NBT/BCIP (nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate) (Boehringer Mannheim, Germany) and then counterstained with Nuclear Fast Red (Vector). As a negative control, each section was processed, but the PCR reaction was performed without AmpliTaqDNA polymerase. Samples from rat bladders instilled with SCH 58500 or a *β-galactosidase*–expressing adenoviral vector (Ad5.β-gal) served as positive and negative controls.

Table 2. Patient Demographics, Tumor Stages, Histologies, and Transgene Expression After SCH 58500 Treatment

Patient No.	Age (years)	Sex	Histology	p53	Stage	Study Group	RT-PCR
001	68	Male	TCC	0	pT4aN2M0 G4	A1	−
002	37	Female	SCC	2	pT3bN0M0 G2-3	A1	−
003	64	Male	TCC	1	pTaN0M0 G2-3	A1	−
004	69	Male	TCC	3	pT1N0M0 G2	B1	+
005	69	Male	TCC	1	pT3aN0M0 G2	B1	+
006	73	Male	TCC	0	pT3aN0M0 G2	B1	+
007	69	Male	TCC	1	pTisN0M0 G3	B2	+
008	69	Male	SCC	3	pT3aN0M0	B2	−
009	70	Male	TCC	0	pT1N0M0 G3	B2	+
010	60	Female	TCC	1	pT1N1M1	B3	ND
011	84	Male	TCC	1	pT2bN1M0 G3	B3	+
012	82	Male	TCC	2	pT4N0M0 G3	B3	+

Abbreviations: TCC, transitional cell carcinoma; SCC, squamous cell carcinoma; Stage, tumor stage according to tumor-node-metastasis classification; p53, immunohistochemical detection of nuclear p53 expression in baseline tumor biopsies in < 10% of tumor cells = 0, 11%-25% of tumor cells = 1, 26%-50% of tumor cells = 2, > 50% of tumor cells = 3; A1, intratumoral injection (part A), dose level 1 (7.5×10^{11} particles); B1, intravesical instillation (part B), dose level 1 (7.5×10^{11} particles); B2, intravesical instillation, dose level 2 (7.5×10^{12} particles); B3, intravesical instillation, dose level 3 (7.5×10^{13} particles); RT-PCR, positive (+) or negative (−) expression of vector-specific *p53* RNA as detected by RT-PCR analysis of samples obtained at cystectomy; ND, not determined (no cystectomy performed).

The protein expression of p53, *p21/WAF1*, apoptosis-related and cell cycle–related genes, and CAR was assessed by immunohistochemistry in formalin-fixed paraffin-embedded tissue sections. Primary antibodies against p53 (M7001, Dako Diagnostika, Hamburg, Germany), *p21/WAF1* (M7202, Dako), Bcl-2 (M0887, Dako), Bak (AM04, Calbiochem, San Diego, CA), Bax (Ab-1/PC66, Calbiochem), MIB1 (dia 505, Dianova, Hamburg, Germany), and CAR (a gift from Dr Robert W. Finberg, Dana-Farber Cancer Institute, Boston, MA[37,38]) were used. Apoptotic cells were visualized by microscopy following the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate-biotin nick end-labeling (TUNEL) method[39] and by means of laser scanning cytometry, as previously described.[40] Normal bladder and tumor tissue samples from patients not treated with SCH 58500 served as controls.

RESULTS

Enrollment and Treatments

Twelve patients from a single center were enrolled onto the study. Baseline characteristics and histologies of the study patients are listed in Table 2. Three patients were treated at dose level 1 in part A (intratumoral injection) of the study. No additional dose escalation was performed in part A. Nine patients were treated at three different dose levels in part B (intravesical instillation). Eleven patients underwent radical cystectomies after study treatment. In one patient, the tumor was determined to be unresectable with curative intent at laparotomy. Thus, tumor samples for assessment of the secondary end points were obtained from 11 patients treated with SCH 58500 at three dose levels.

Toxicity

Postoperatively, one patient treated in part A suffered from WHO grade 1 fatigue. No toxicities were observed in the other two patients treated with intratumoral injection at cystoscopy. The predominant toxicities observed in patients treated in part B of the study were urethral and vesical burning, which reached WHO grade 2 in two patients and WHO grade 3 in another two patients. In addition, one patient each experienced WHO grade 2 and grade 3 abdominal pain. These symptoms were relieved in two patients treated at dose level 1 by a reduction of the contact time, for which the transurethral catheters were clamped. Hence, for patients treated at dose levels 2 and 3, the treatment was administered in two sequential 30-minute sessions. Additionally, patients treated at dose level 3 were premedicated with 50 mg of pethidine and 20 mg of butylscopolamine. Despite these modifications, the planned contact time had to be reduced by several minutes in three patients treated at dose level 2 and in one patient treated at dose level 3. All symptoms resolved immediately after release of the transurethral catheter and bladder irrigation with saline. No fever, chills, or other signs of systemic toxicity were observed in patients treated in part B. No alterations of laboratory parameters, including liver enzymes and bilirubin, were detected before surgery on day 3. Three patients were hospitalized because of fever of unknown origin within 4 to 6 weeks after surgery and quickly recovered under treatment with broad-spectrum antibiotics. In one of these patients, a methicillin-resistant *Staphylococcus aureus* was isolated from a catheter. Thus, even at the highest dose level of 7.5×10^{13} particles SCH 58500 administered by intravesical instillation, no dose-limiting toxicities were observed.

Transgene Expression and Biologic Activity

In two of three assessable patients treated in part A (intratumoral injection), vector DNA was found by PCR

Table 3. Induction of p21/WAF1 RNA and Protein Expression After Intravesical SCH 58500 Treatment

Patient No.	Study Group	Normal Bladder Tissue		Tumor Tissue	
		p21 RNA	p21 IHC	p21 RNA	p21 IHC
Controls	—	1.08 ± 1.8	—	0.26 ± 0.38	—
004	B1	3.5 ± 2.2	0/0	0.92 ± 1.12	0/2
005	B1	2.47	0/0	1.16 ± 1.37	0/2
006	B1	.57 ± 0.4	0/0	1.16 ± 1.43	0/0
007	B2	2.62	0/0	0.32 ± 0.29	0/0
008	B2	ND	0/0	0.32 ± 0.29	0/0
009	B2	4.59	0/0	0.61	0/0
010	B3	ND	ND	ND	ND
011	B3	1.29 ± .42	0/0	10.4 ± 20.69	0/2
012	B3	2.66 ± 1.56	0/0	3.99 ± 4.7	0/1

Abbreviation: ND denotes not determined (insufficient sampling or no cystectomy performed).

NOTE. Tissue samples from bladder tumors and normal bladder tissue obtained at cystectomy were examined by real-time RT-PCR (p21 RNA) and immunohistochemistry (p21 IHC). Normal bladder samples from four patients and tumor samples from five patients not treated with SCH 58500 served as controls for real-time RT-PCR (Controls). Results from real-time RT-PCR are expressed as mean ± SD × 10,000 copies normalized to 1,000 copies *GAPDH* RNA. Results from immunohistochemistry are presented as nuclear expression of p21/WAF1 in biopsies before and after SCH 58500 treatment (< 10% of tumor cells = 0; 11%-25% of tumor cells = 1; 26%-50% of tumor cells = 2; > 50% of tumor cells = 3).

analysis of posttreatment tumor samples (not shown). However, no transgene expression as assessed by RT-PCR analysis of vector-specific *p53* expression was detected after intratumoral injection of SCH 58500 at cystoscopy (Table 2). In contrast, vector-specific *p53* transgene expression was found by RT-PCR analyses of tissue samples from seven of eight assessable patients treated with intravesical instillation of SCH 58500 (Table 2).

To address whether the *p53* transgene expression translated into biologic activity, we determined the quantitative expression of the p53 target gene *p21/WAF1* by real-time RT-PCR analysis of tumor and normal bladder samples from patients treated with intravesical instillation of SCH 58500 or untreated control patients. The *p21/WAF1* expression in tumor samples from untreated control patients was lower than in normal bladder samples (Table 3). Assaying nontumor bladder samples from patients treated with SCH 58500 instillation, moderate changes in *p21/WAF1* expression were detected when compared with untreated controls (Table 3). However, in tumor samples from patients treated at the highest dose level of 7.5×10^{13} particles SCH 58500 *p21/WAF1*, expression was increased up to 40-fold compared with control tumor samples from patients not receiving gene therapy (Table 3). Immunohistochemical analyses revealed an increased *p21/WAF1* protein expression after SCH 58500 treatment in tumor tissues but not in normal bladder samples from four patients with undetectable or low *p21/WAF1* protein expression at baseline (Table 3). No significant correlation between transgene expression, *p21/WAF1* induction, and CAR expression, as determined by RT-PCR analysis and immunohistochemistry, could be established. However, the CAR expression detected by immunohistochemistry exhibited a considerable heterogeneity among tumors from different patients as well as among different regions of the same tumor (not shown). Immunohistochemical analyses of p53 expression or expression of additional apoptosis-related or cell cycle–related genes revealed no consistent changes in relation to SCH 58500 treatment. Moreover, we failed to detect a significant induction of apoptosis as assessed by TUNEL staining and microscopy or laser scanning microscopy in samples taken at cystectomy approximately 48 hours after SCH 58500 treatment (not shown).

Taken together, these data demonstrate that a detectable p53 transgene expression in bladder tumors can be achieved by intravesical instillation of SCH 58500 in combination with a transduction-enhancing agent. At the highest dose level of 7.5×10^{13} particles SCH 58500, evidence for biologic activity in terms of RNA and protein expression of the p53 target gene *p21/WAF1* was obtained.

Vector Distribution

Using quantitative real-time PCR, SCH 58500 DNA copies were detected in normal bladder and tumor samples from patients treated with intravesical instillation in a dose-dependent manner, whereas no SCH 58500 DNA was found in samples from control patients not treated with SCH 58500 (Fig 1). The demonstration of vector DNA or transgene expression in tissue homogenates does not provide information regarding the transduction efficacy or the vector penetration. Therefore, tissue sections from patients treated in part B were analyzed by in situ PCR, revealing a strong vector-specific signal throughout the whole urothelium (Fig 2). Moreover, SCH 58500 DNA was also detected

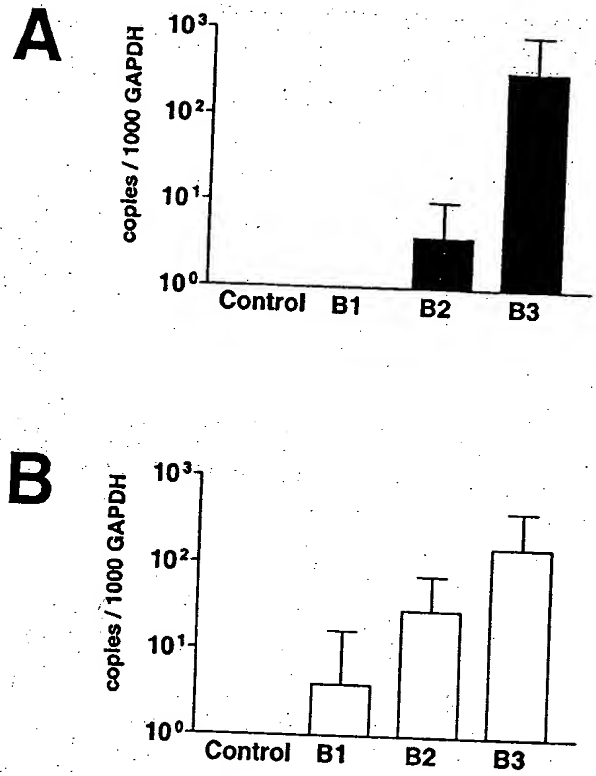

Fig 1. Quantitative detection of SCH 58500-specific DNA sequences (mean ± SD) by real-time PCR analysis of samples from tumor (A) and nontumor bladder tissue (B) of untreated bladder cancer patients (control) and patients treated with intravesical instillation of SCH 58500 at dose levels 1 (B1), 2 (B2), and 3 (B3).

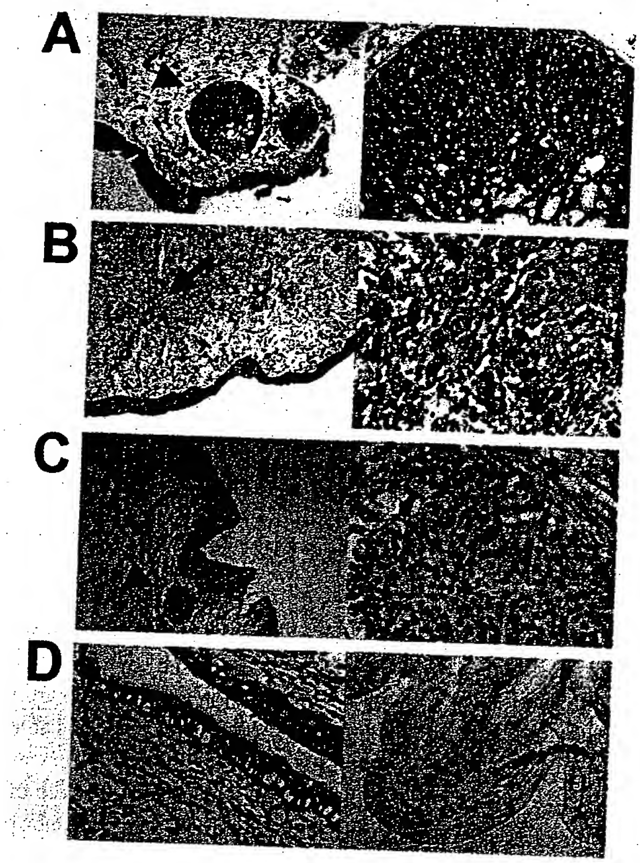

Fig 2. SCH 58500 vector distribution (in situ PCR) in tissue sections from patient no. 011 (A and B) and patient no. 005 (C) treated with intravesical instillation. Arrowheads indicate submucosal tumor nodules (A and C); arrow indicates cells in the Lamina propria (B). Sections from rat bladders injected with SCH 58500 (D, left panel) or control virus (D, right panel) are shown as positive and negative controls.

in submucosal tumor nodules as well as in cells in the Lamina propria. Thus, intravesical instillation of SCH 58500 in combination with a transduction-enhancing agent can achieve an uniform vector penetration throughout the urothelium as well as into submucosal tumor tissues.

Virologic Studies

After SCH 58500 treatment, all patients in both study groups underwent extensive bladder irrigation with 6 L saline through a transurethral catheter over a period of 36 to 48 hours. Excretion of infectious adenoviruses was detected by a sensitive flow cytometry-based assay[30] in samples taken from the first 2 to 4 L of void volume. No detectable urinary adenovirus excretion was found after 6 L of bladder irrigation (Fig 3). None of the urine samples taken 24 hours after study treatment gave a positive result in the qualitative on-site ELISA assay (not shown).

Long-Term Follow Up

Nine of the 12 study patients were alive at a median follow-up of 30 months. In addition to SCH 58500 treatment and radical cystectomy, two patients received a platinum-based adjuvant chemotherapy regimen. In one patient treated in part A, fulminant liver metastases developed 4 weeks after surgery, which were not detectable on computed tomogram and ultrasound examinations performed at the preoperative staging. The patient was treated with palliative chemotherapy, but he died from progressive liver failure 7 weeks after cystectomy. One patient treated in part A developed a *Mycoplasma* pneumonia during adjuvant chemotherapy. In total, three patients died from disease progression, and one patient is being treated with palliative chemotherapy for recurrent disease.

DISCUSSION

A major challenge in the conservative management of localized bladder cancer is the frequent recurrence and progression to an advanced tumor stage in patients with high-risk tumors.[2] To improve disease control, local tumor

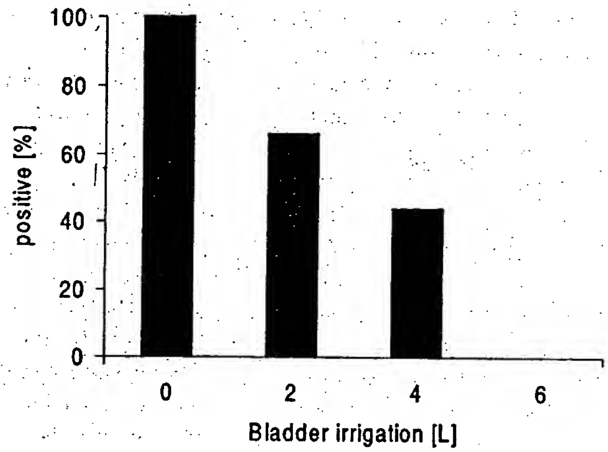

Fig 3. Excretion of infectious adenoviruses after intravesical SCH 58500 treatment. The percentage of patients ($n = 12$) with urine samples positive for infectious adenoviruses after the indicated volumes of bladder irrigation with saline.

resection is combined with intravesical therapy with bacillus Calmette-Guerin or anticancer agents.[8] However, these treatments have a substantial toxicity and may reduce the risk of recurrences but do not prevent disease progression.[6,7] Thus, new treatment options for high-risk superficial bladder cancer are required. Mutations of the *p53* tumor suppressor gene are frequently found in bladder cancer, are associated with an adverse prognosis in some studies, and may contribute to a more aggressive clinical course and resistance to anticancer treatment.[14,15] In an ortopic injection model of bladder cancer, *p53* gene transfer acted synergistically with cisplatin to prevent tumor growth and induce apoptosis in vivo.[41]

In the present phase I study, we tested whether adenoviral vector-mediated wild-type *p53* gene transfer is safe, feasible, and biologically active in patients with invasive bladder cancer. Taking advantage of the anatomy of the bladder, we planned to evaluate two different modes of vector administration: the intratumoral injection of vector solution, because it has been performed in several clinical studies of cancer gene therapy,[19-21] and the intravesical vector instillation via a transurethral catheter. Because preclinical studies convincingly demonstrated that the transduction efficacy of adenoviral vectors instilled into the bladder can be dramatically enhanced by the addition of several compounds,[27,28] here we administered intravesical SCH 58500 in combination with the transduction-enhancing agent Big CHAP.[28] Both modes of administration of the study treatment, intratumoral injection at cystoscopy and transurethral intravesical instillation, were well tolerated and devoid of any detectable systemic toxicity. Successful gene transfer after intravesical instillation of SCH 58500 in combination with Big CHAP was detected by RT-PCR analysis in seven of eight assessable patients. Moreover, evidence for biologic activity of the transgene, as determined by quantitative RT-PCR analysis of RNA expression as well as by immunohistochemical analysis of protein expression of the p53 target gene *p21/WAF1*,[32] was found in patients treated at higher dose levels. Transgene expression did not seem to correlate with the CAR expression status of the tumor samples as determined by RT-PCR analysis and immunohistochemistry. However, the relatively small number of patients enrolled onto this study and the detection methods for CAR expression might have influenced this result. Compared with the effective transduction achieved by intravesical vector instillation, no evidence for transgene expression was detected in the three patients treated by intratumoral injection of SCH 58500 at dose level 1, whereas SCH 58500 DNA sequences were detectable in two patients by PCR analysis. This was surprising, given that in a previous study in patients with NSCLC treated by intratumoral injection of SCH 58500, *p53* transgene expression was detected in four of five assessable patients receiving the same vector dose of 7.5×10^{11} particles.[20] Because intratumoral vector injection at cystoscopy is a relatively invasive procedure compared with transurethral vector instillation, it was decided not to proceed with the dose escalation in part A of this trial. Hence, we cannot exclude that at higher dose levels, a *p53* transgene expression in bladder tumors would have been achieved by intratumoral injection of SCH 58500 at cystoscopy. Furthermore, the addition of Big CHAP or other transduction-enhancing agents[28] might also be beneficial in the case of intratumoral vector injection in the bladder. However, in the light of the efficacy and ease of the intravesical instillation approach, intratumoral vector injection at cystoscopy clearly is the inferior approach for vector administration in bladder cancer.

In contrast to the results obtained with the p53 target gene *p21/WAF1*, we found no consistent changes in the expression of *p53*, various cell cycle–related or apoptosis-related genes, or TUNEL staining in response to SCH 58500 administration. This observation might be limited by the small number of patients enrolled onto the trial and the availability of only a single time point for these examinations. Moreover, the activity of many genes regulating apoptosis is not controlled by their expression level but by conformational changes or changes in their subcellular localization,[42] which cannot be detected by the methods applied in this study. Nevertheless, the *p21/WAF1* response is a valid marker for biologic activity of transgenic p53,

which has been confirmed in additional settings of clinical p53 gene therapy.[35,43]

In addition to molecular and immunohistochemical evidence for transgene expression and biologic activity, important information related to the vector distribution throughout the bladder and vector penetration into tumor tissues was gathered from this trial. We demonstrated by quantitative PCR analysis that administration of higher particle doses resulted in the recovery of higher copy numbers of SCH 58500-specific DNA from tissue samples (Fig 3). This was not unexpected; however, it suggests that together with the evidence for increased *p21/WAF1* expression at high doses, a plateau of the biologic activity was not reached by the intravesical instillation of 7.5×10^{13} particles SCH 58500. Presently, technical limitations preclude the administration of a more concentrated adenovirus solution, leaving this issue unresolved. With respect to the vector distribution after intravesical instillation, we found a uniform distribution of SCH 58500 DNA throughout the normal urothelium and the luminal tumor tissues by in situ PCR analysis of bladder sections. Moreover, vector DNA could also be found in apparently submucosal tumor nodules as well as in cells in the Lamina propria. These results confirm the hypothesis that the instillation approach results in an improved vector distribution. In addition, they demonstrate that even submucosal tumor cells can be targeted by the luminal administration of an adenovirus in combination with a transduction-enhancing agent in the bladder.

The optimal dosing schedule for intravesical SCH 58500 instillation remains to be established. Because of the procedure-associated discomfort observed in most patients treated in part B of this study, the contact times varied considerably. Yet SCH 58500 penetration and transgene expression analyses yielded promising results. It seems likely that even shorter contact times than the ones allowed in the course of this trial might result in sufficient transduction rates with lower local toxicity, a hypothesis supported by initial data from preclinical in vivo models. The intravesical instillation of SCH 58500 through a transurethral catheter also is environmentally safe, because infectious adenoviruses excreted in the urine after SCH 58500 treatment can easily be recovered in a contained system. The virologic studies performed in this trial suggest that if the bladder is sufficiently irrigated, infectious viral particles are only excreted with the first 4 L of irrigation fluid. This could minimize hospitalizations and could even allow outpatient treatment.

The design of the present phase I study precluded the collection of data regarding the long-term effects of the intravesical administration of such high-vector doses as well as signs for clinical efficacy. However, important and unique data demonstrating effective vector distribution, transgene expression, and biologic activity after a clinically practicable and safe gene transfer procedure were obtained in patients with invasive bladder cancer. These results provide a strong rationale for future investigation of adenovirus therapy in bladder cancer and support trials addressing the clinical efficacy of intravesical SCH 58500 treatment in patients with superficial high-risk bladder cancer.

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